

## Host-Pathogen Interactions

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### INNOVATIVE ANTI-MICROBIAL STRATEGIES AGAINST BURULI ULCER: BACTERIOPHAGE THERAPY

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Buruli Ulcer (BU), caused by *Mycobacterium ulcerans*, is an emerging necrotizing skin disease. Currently, antibiotherapy with rifampicin and streptomycin is recommended by the WHO, but in extensive ulcerative lesions, surgical resection of the infected skin is still necessary. Bacteriophages and their lysins (Lys) are a class of antimicrobial agents that have been regarded as an alternative method to control bacterial infections. The overall goal of this study is to evaluate the efficacy of mycophages and Lys in the control of *M. ulcerans*, as a novel therapeutic approach against BU.

After establishing the antimycobacterial activity of mycobacteriophage D29 *in vitro*, we further tested its efficacy *in vivo*. For that mice were injected s.c. with a single dose of mycophage D29 in infected footpads, at an advanced stage of infection. The efficacy of phage treatment was evaluated by footpad swelling and viable *M. ulcerans* growth. We show that a single injection of mycophage D29 can effectively decrease *M. ulcerans* proliferation and prevent footpad ulceration.

Additionally, we wanted to determine whether mycophage Lys were effective in controlling *M. ulcerans* proliferation. For that, Lys were expressed in an *E. coli* BL21(DE3)(pET) system and purified by an affinity chromatography. In order to evaluate the antimicrobial activity of Lys, a lysoplate assay was performed with *M. ulcerans* isolates from endemic BU areas. Our results show that Lys has lytic activity *in vitro* against *M. ulcerans* isolates, in a dose dependent manner as demonstrated by induced spots in bacterial lawns.

Our next step was to evaluate the bioavailability and cytotoxicity of Lys *in vivo*. At different time points after s.c. Lys injection in the footpad, the presence of protein in tissue supernatant and in the serum of mice was detected by western blotting. Histological analysis was performed on footpad tissue sections stained with hematoxylin and eosin. We observed no significant alterations in the footpad of treated mice, showing that Lys is not cytotoxic *in vivo*. Importantly, our results show an enzymatic activity associated with the detection of Lys in footpads of mice, at least 6h after s.c. injection.

After these promising results our research is now focused on therapeutic studies to test Lys activity *in vivo* against *M. ulcerans*. One possible approach to improve Lys bioavailability could be the association of Lys with a drug delivery system with topical application.